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Project Report: Genomes of Permafrost Bacteria

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Project Progress

A major objective of our research team is to determine the repertoire of genes necessary for bacteria to live in the permafrost environment. Central to this effort is determining (in collaboration with the Joint Genome Institute and the Lawrence Livermore National Laboratory) the complete genome sequences of two bacteria that we have isolated from Siberian permafrost: *Psychrobacter* strain 273–4 and *Exiguobacterium* strain 255–15, psychrophilic bacteria isolated from permafrost soils thought to have been constantly frozen for some 40,000 and 3 million years, respectively.

Sequencing of the *Psychrobacter* genome has been completed. Considerable effort over the past year went into a “manual curation” of each open reading frame (ORF) to yield the first completely annotated genome of a psychrophilic microorganism. A number of interesting findings have emerged. The genome is 2.64–megabases in size, contains 2,147 ORFs including a 20,145 base pair (bp) tandem repeat that encodes an extremely large hypothetical protein of 6,715 amino acids. The genome encodes four histone–like proteins that potentially have roles in cold tolerance as well as enzymes for a complete glyoxylate shunt pathway enabling growth of an acetate. Analysis of genes that were found to be up–regulated in response to low temperature (see transcriptome and proteome projects) revealed the existence of a novel 139 bp sequence present 294 times in the genome that may have roles in cold–regulated gene expression. Phylogenetic analysis has also revealed that an isocitrate lyase encoded by *Psychrobacter* forms a unique cluster with cold–induced isocitrate lyases of the psychrophile *Colwellia maris* and three other microbes that grow at low temperatures. Significantly, this cluster of sequences contains three insertions that may increase flexibility of the enzymes and reduce activation energy, an important factor for function at low temperature.

Significant progress has also been made in sequencing the *Exiguobacterium* 255–15 genome, estimated to be about 2.9 Mb in size. Currently, the sequences are assembled into 70 contigs predicted to encode 2,977 proteins.

Approximately 75% of the *Exiguobacterium* ORFs encode putative homologs of *Psychrobacter* proteins and there appear to be many ORFs that encode putative homologues of genes from *Bacillus* and other genera that are known to be expressed at cold temperatures including proteins involved in ribosomal binding, membrane alteration, metabolism, DNA replication, and transcription.

Highlights

- Completed sequencing and annotating the first genome of a psychroactive microbe, *Psychrobacter* strain 273–4.
- Found evidence that some of the genes in this genome have evolved to be active at low temperatures.
- Unique repeat sequence was found in the genome.
- Genome of *Exiguobacterium* strain 255–15, an ancient gram–positive microbe, is nearing completion.

Roadmap Objectives

- **Objective No. 5.1:** Environment–dependent, molecular evolution in microorganisms
- **Objective No. 5.3:** Biochemical adaptation to extreme environments
- **Objective No. 6.2:** Adaptation and evolution of life beyond Earth